P-selectin plays a role in haem-induced acute lung injury in sickle mice

Acute chest syndrome (ACS) is a major pulmonary complication and a leading cause of death in sickle cell disease (SCD) (Gladwin & Vichinsky, 2008). Extracellular haem has been shown to be independently associated with ACS and vaso-occlusive crisis (VOC). Severe ACS is frequently preceded by painful VOC and acute intravascular haemolysis (Vichinsky et al., 2000). A rapid drop in baseline haemoglobin (mean decrease of 7-8 g/l) and elevated lactate dehydrogenase reported during ACS development indicates a cardinal role of acute intravascular haemolysis in severe ACS (Vichinsky et al., 1997). Accordingly, infusion of purified haem causes respiratory failure and lethal acute lung injury (ALI) reminiscent of severe ACS in transgenic SCD (SS) mice (Ghosh et al., 2013).

P-selectin, a cell adhesion molecule expressed on activated endothelium and platelets, is implicated in obstructing microvascular flow, causing VOC (Embry et al., 2004) by promoting adhesion of sickle erythrocytes and leucocytes to the endothelial wall (Matsui et al., 2001). Extracellular haem activates endothelial P-selectin expression, inducing vascular stasis in murine models of SCD (Belcher et al., 2014). A recent trial showed that an anti-human P-selectin antibody reduced the frequency of painful events in SCD patients (Ataga et al., 2017), although it could not determine the role of P-selectin in ACS. This study addresses this knowledge gap and provides mechanistic evidence demonstrating a pathogenic role for P-selectin in the development of haem-induced ALI in sickle mice.

To verify the involvement of P-selectin in ACS, we infused a function-blocking monoclonal murine anti-P selectin antibody (RB 4034; Millipore, Burlington, MA; 2 mg/kg) or control IgG into SS mice followed by induction of ALI using purified haemin (70 mol/kg) (Fig. 1A). Mortality data showing anti-P-selectin antibody (ab) prevents haem-induced death in SS mice (*P < 0.05; Mantel-Cox test). (B) Real time monitoring of arterial oxygen saturation (% SpO2) indicating hypoxaemia in control IgG treated mice, while antibody-treated mice had no oxygen desaturation. (C) Representative histopathology of lung tissue from mice 2 h after haem challenge. The haematoxylin and eosin stained sections under different magnification (40× and 100×) clearly show protection from lung damage following antibody blocking of P-selectin in SS mice. (D) Assessment of lung injury, using gravimetric analysis, in SS mice after indicated treatment and haem challenge. [Colour figure can be viewed at wileyonlinelibrary.com]

Fig 1. Functional P-selectin is essential for haem induced acute chest syndrome in sickle mice. Wild-type Townes' sickle mice (SSWT) given intravenous injection of anti-P-selectin antibody or control IgG (n = 6), 30 min prior to infusion of purified haemin (70 μmol/kg). (A) Mortality data showing anti-P-selectin antibody (ab) prevents haem-induced death in SS mice (P < 0.05; Mantel-Cox test). (B) Real time monitoring of arterial oxygen saturation (% SpO2) indicating hypoxaemia in control IgG treated mice, while antibody-treated mice had no oxygen desaturation. (C) Representative histopathology of lung tissue from mice 2 h after haem challenge. The haematoxylin and eosin stained sections under different magnification (40× and 100×) clearly show protection from lung damage following antibody blocking of P-selectin in SS mice. (D) Assessment of lung injury, using gravimetric analysis, in SS mice after indicated treatment and haem challenge. [Colour figure can be viewed at wileyonlinelibrary.com]

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purified haem (70 \(\mu\)mol/kg) as previously described (Ghosh et al., 2013). All experimental mice were monitored for real-time arterial oxygen saturation (\% \(\text{SpO}_2\)) using the MouseOx\textsuperscript{TM} pulse-oximeter (Starr Life Sciences, Oakmont, PA) and the lung wet/dry weight ratio was determined at the end of the experiment. Five of six SS mice pre-treated with the anti-P-selectin antibody did not develop lung injury while all six SS mice pre-treated with the IgG control succumbed (\(P = 0.03\)), with severe hypoxaemia, post-mortem evidence of alveolar flooding and extensive lung damage (Fig 1A–D). A drop in the platelet count is a predictor of respiratory failure in ACS patients (Chaturvedi et al., 2016). In this study,
we found that the platelet count in IgG control group dropped significantly, whereas it remained unaltered in SS mice treated with anti-P-selectin antibody (Figure S1). Infusion of a higher concentration of haem (210 μmol/kg bw) triggered a lethal ALI in wild type C57BL/6J mice (P-Sel+/+; B6; JAX stock# 000664), while haem did not cause ALI in congenic P-selectin global knockout mice (P-Sel−/−; B6.129S7-Selpm1Bay/j; JAX stock# 002289) (Figure S2).

To determine whether platelet P-selectin (in the haematopoietic compartment) or P-selectin expressed by the endothelium (non-haematopoietic compartment) played the dominant role in promoting ALI, we generated bone marrow chimeric C57BL/6 mice lacking P-selectin in haematopoietic (P-SelPLT−/−) and non-haematopoietic (P-SelEC−/−) compartments. For this purpose, we transplanted whole bone marrow cells from P-Sel+/+ or P-Sel−/− donor mice to P-Sel−/− and P-Sel+/+ recipient mice, generating P-SelBC−/− and P-SelPLT−/− mice, respectively. Three of the four P-SelBC−/− mice studied were protected while all three P-SelPLT−/− congenic control mice studied succumbed to the haem infusions. Lung injury in the congenic B6 P-SelPLT−/− mice was characterized by severe hypoxemia (SpO2: 82.75 ± 2.14%) and oedema (Fig 2A–D). Next, we generated bone marrow chimeric SS mice that lack endothelial P-selectin (SSP-SelEC−/−) or express endothelial P-selectin (SSP-SelEC+/+) by transplanting congenic P-Sel−/− and P-Sel+/+ mice with SS mouse bone marrow. Induction of ACS resulted in lethality in 3 of seven SSP-SelEC−/− mice and in all seven SSP-SelEC+/+ mice (Fig 2E). The SSP-SelEC−/− mice that died in this experiment experienced similar degree of lung damage, suggesting a role for other factors in this haemolytic ALI model (Fig 2E–H). Haem infusion results in de novo haem release in SS mice during development of acute respiratory failure (Ghosh et al., 2013), suggesting that continuous intravascular haemolysis feeds the inflammation in ACS. In this study, we found that SSP-SelEC−/− cleared ~70% of the haem bolus within 25 min, while the total plasma haem (TPH) increased over 2-fold in the congenic SSP-SelEC+/+ mice. Sequential TPH measurement in SS mice pre-treated with either anti-P-selectin antibody or IgG control revealed rapid clearance of the haem in the anti-P-selectin antibody-treated mice, while TPH was amplified IgG control littersmates (Figure S3). Together these results indicate that P-selectin potentiates extracellular haem-induced inflammation by releasing more haem into the circulation, most likely via a mechanism involving enhanced adhesions of sickle erythrocytes.

P-selectin has been identified as a key player in acute vascular occlusion in SCD (Chang et al., 2010). A recent clinical trial showed that intermittent intravenous injections of human anti-P-selectin antibody over a period of 52 weeks reduced rates of pain crises in patients with SCD (Ataga et al., 2017). However, there were not enough ACS incidents to evaluate the efficacy of this treatment in ACS. Here we propose a model of ACS pathogenesis in which activation of endothelial P-selectin promotes occlusion and subsequent lysis of sickle erythrocytes yielding supra-physiologically high local concentrations of haem, that disrupts the alveolar capillary barrier to cause alveolar flooding, hypoxaemia and respiratory failure. The anti-P-selectin antibody blocks P-selectin adhesion, which would inhibit the entrapment of sickle cells to promote de novo release of extracellular haem critical to sustaining the inflammation in ACS. This assertion is based on the finding that SSP-SelEC−/− mice cleared ~70% of the haem within the period of the experiment. On the contrary, total plasma haem increased over 2-fold in the SSP-SelEC+/+ mice. Inhibiting P-selectin adhesion may be sufficient to thwart this process and prevent and potentially treat ACS. Finally, this study provides proof-of-principle that anti-P-selectin antibodies can be used to block ACS development.

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Authorship contributions

SG, BF and FW performed experiments and organised the data. SFOA conceived the study, and wrote the manuscript with SG.

Disclosure of Conflicts of Interest

The authors have declared that no conflict of interest exists.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Drop in platelet is evident during heme induced acute lung injury.

Figure S2. P-selectin is essential for heme induced acute lung injury.

Figure S3. Endothelial P-selectin involves in attenuation of excess of extracellular heme release following heme challenge in sickle mice.
References


